AD_____
(Leave blank)

Award Number:
W81XWH-08-1-0382

TITLE:
Correlating MALDI and MRI Biomarkers of Breast Cancer

PRINCIPAL INVESTIGATOR:
Amelie R. Gillman

CONTRACTING ORGANIZATION:
Vanderbilt University
Nashville, TN 37232-2310

REPORT DATE:
August 2009

TYPE OF REPORT:
Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 0704-0188*



| **1. REPORT DATE** *(DD-MM-YYYY)* | **2. REPORT TYPE** | **3. DATES COVERED** *(From - To)* |
|---|---|---|
| 31-08-2009 | Annual Summary | 1 July 2008 – 30 Jun 2009 |

**4. TITLE AND SUBTITLE**
Correlating MALDI and MRI Biomarkers of Breast Cancer

**5a. CONTRACT NUMBER**
W81XWH-08-1-0382

**5b. GRANT NUMBER**

**5c. PROGRAM ELEMENT NUMBER**

**6. AUTHOR(S)**
Amelie R. Gillman
Email: amelie.r.gillman@vanderbilt.edu

**5d. PROJECT NUMBER**

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**

Vanderbilt University
Institute of Imaging Science
Medical Center North, AA-1105
Nashville, TN 37232-2310

**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

**10. SPONSOR/MONITOR'S ACRONYM(S)**

**11. SPONSOR/MONITOR'S REPORT NUMBER(S)**

**12. DISTRIBUTION / AVAILABILITY STATEMENT**
Approved for public release; distribution unlimited

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**
The processes of tumor growth and treatment response are associated with the upregulation of numerous proteins, yet current clinical imaging methods of cancer characterization monitor only gross morphology. This study combines *in vivo* diffusion-weighted magnetic resonance imaging (MRI) with matrix-assisted laser desorption ionization (MALDI) analysis of healthy and tumorous *ex vivo* specimens in order to examine the proteomic influences on the apparent diffusion coefficient (ADC) provided by MRI. ADC and MALDI data were acquired in a rat model of C6 glioma brain cancer. Principal component analysis was applied to the MALDI data to generate eigenimages which represent the relative concentration of MALDI data in space. The ADCs and eigenimage intensities were compared in selected anatomical regions using the linear Pearson correlation coefficient. ADC and eigenimage intensity correlated significantly (p-value less than or equal to 0.05) in 44.0% of the comparisons conducted in this study. Similar ADC populations within specific tissue types correlated with qualitatively similar protein profiles in controls, but less similar protein signatures in tumor rats. The methods developed in this study constitute a novel approach for identification of correlations between non-invasive MR measurements and their underlying molecular-level contrast sources. Moreover, this work represents a basic yet vital step towards the long-term objective of facilitating clinical assessment of tumor status via non-invasive imaging techniques.

**15. SUBJECT TERMS**
MRI, MALDI, cancer, principal component analysis, proteins, correlation

| **16. SECURITY CLASSIFICATION OF:** | | | **17. LIMITATION OF ABSTRACT** | **18. NUMBER OF PAGES** | **19a. NAME OF RESPONSIBLE PERSON** |
|---|---|---|---|---|---|
| **a. REPORT** | **b. ABSTRACT** | **c. THIS PAGE** | | | USAMRMC |
| U | U | U | UU | 10 | **19b. TELEPHONE NUMBER** *(include area code)* |

## Table of Contents

## INTRODUCTION

This report summarizes the work and results of the first of three years of predoctoral training funded by this grant. This grant supports the PI's preparation for a career in breast cancer research by providing for training in image processing, analytical methods, and state-of-the-art laboratory techniques used in cancer research. The purpose of the funded research is to assess the correlation between physiological parameters reported by magnetic resonance (MR) imaging and tumor protein distribution determined from matrix-assisted laser desorption ionization (MALDI) mass spectrometry measurements. The overarching goal of this work is to elucidate the proteomic influences of contrast in MR cancer imaging in an effort to improve clinical breast cancer care by enabling clinicians to quickly establish or modify treatment regimens based on non-invasive assessment of cellular-level tumor status.

## BODY

The training plan proposed in the approved Statement of Work includes didactic coursework, laboratory training, and cultivation of a broad-based knowledge of contemporary breast cancer and imaging science issues via attendance of relevant seminars and conferences. Progress towards completion of the training plan includes fulfillment of didactic coursework, research, and thesis requirements for attainment of the degree of Master of Science from the Vanderbilt University Graduate School. In addition, the PI has received laboratory training on all equipment utilized in the study. Development of a wide-ranging knowledge base of contemporary breast cancer and imaging science issues is ongoing and facilitated by regular seminars held throughout the year at Vanderbilt University and elsewhere.

The research plan proposed in the approved Statement of Work during the first award year includes refinement and validation of data acquisition and analysis processes in an animal model of brain cancer. The PI has worked closely with Vanderbilt University Institute of Imaging Science (VUIIS) faculty mentors to develop computational techniques for three-dimensional reconstruction and co-registration of MR and MALDI data via processes described in [1]. Analytical methods which have been used to examine the relationship between protein profiles and diffusion metrics in the co-registered data obtained from the implemented animal model include principal component analysis [2], linear regressive analysis, and correlation analysis based on the linear Pearson correlation at the 95% confidence level [3].

## KEY RESEARCH ACCOMPLISHMENTS

Key research accomplishments emanating from this training grant include the following:

- completion of graduate-level didactic coursework to support breast cancer imaging research (e.g., Cancer Imaging, Quantitative Magnetic Resonance Imaging, and Medical Image Registration classes)
- completion of laboratory training on a 9.4 T Varian Inova MR scanner
- completion of laboratory training on a Leica CM3600 Cryomacrotome
- completion of laboratory training on an Autoflex III Bruker Daltonics linear MALDI time-of-flight mass spectrometer

- additional supportive training via attendance of regular seminars sponsored by the Vanderbilt University Institute of Imaging Science, the Vanderbilt University Medical Center, and multiple academic departments

- refinement and validation of three-dimensional reconstruction and inter-slice registration of specimen volumes from MALDI data on multiple two-dimensional specimen slices

- refinement and validation of methods for co-registration of MR and MALDI datasets

- application of principal component analysis to hybrid MALDI/MRI data sets to identify multi-spectral basis sets

- application of linear regressive and correlation analysis techniques to determine the relationship between proteomic and diffusion metrics

## REPORTABLE OUTCOMES

The reportable outcomes that have resulted from this training grant include the following:

- attainment of the degree of Master of Science in the field of Biomedical Engineering from the Vanderbilt University Graduate School

- presentation of research methods and results to the VUIIS faculty, staff, and trainees at the 2009 annual VUIIS Research Retreat [4]

## CONCLUSION

Several hundred regions of interest examined in co-registered MALDI/MR datasets obtained from multiple animals have exhibited a significant linear correlation between protein signature intensity and apparent diffusion coefficient (ADC) in both healthy and tumorous tissues. A representative ADC map is shown in Figure 1 of the appendix. Regions of interest in a hybrid MALDI/MR dataset are depicted in Figures 2 and 3 of the appendix [5]. Figure 4 of the appendix conveys the relationship between the two metrics on a voxel-by-voxel basis in a selected region of interest. Overall, results of research conducted during the first award year provide strong support for the hypothesis that protein content may significantly affect contrast in diffusion-weighted MR imaging. In addition to further examination of the correlation between diffusion metrics and protein signatures in cancerous tissue, future work will incorporate multi-parametric MR data into the analysis. Based on the extensive positive correlation results obtained during the first year of this study, it is expected that multivariate regressive analysis of co-registered protein profiles and data acquired from multiple specialized MR methods will establish a comprehensive characterization of the contribution of molecular-level tissue components to the signal contrast observed in MR data. Thus, the information generated by the work funded by this training grant constitutes a basic yet vital step towards the long-term objective of facilitating clinical assessment of tumor status via non-invasive imaging techniques.

REFERENCES


1. Sinha T, Khatib-Shahidi S, Yankeelov T, Mapara K, Ehtesham M, Cornett D, Dawant B, Caprioli R, Gore J. Integrating Spatially Resolved Three Dimensional MALDI IMS with *in vivo* Magnetic Resonance Imaging. *Nature Methods*, 2008; 5(1): 57 – 59.

2. Van de Plas R, Ojeda F, Dewil M, Van Den Bosch L, De Moor B, Waelkens E. Prospective Exploration of Biochemical Tissue Composition *via* Imaging Mass Spectrometry Guided By Principal Component Analysis. *Pac. Symp. Biocomp*. 2007; 12: 458 – 69.

3. Zou K, Tuncali K, Silverman S. Correlation and Simple Linear Regression. *Radiology* 2003; 227:617 – 628.

4. Gillman, A. (2009) Correlating MALDI-IMS and MRI Diffusion Measurements in the C6 Rat Glioma Tumor Model. *Presented at the Vanderbilt University Institute of Imaging Science Annual Research Retreat*. Knoxville, TN.

5. Paxinos G, Watson, C. *The Rat Brain in Stereotaxic Coordinates.* 4th ed. San Diego: Academic Press, 1998.

APPENDIX: SUPPLEMENTARY FIGURES

The following figures illustrate representative data and results as described in the text.

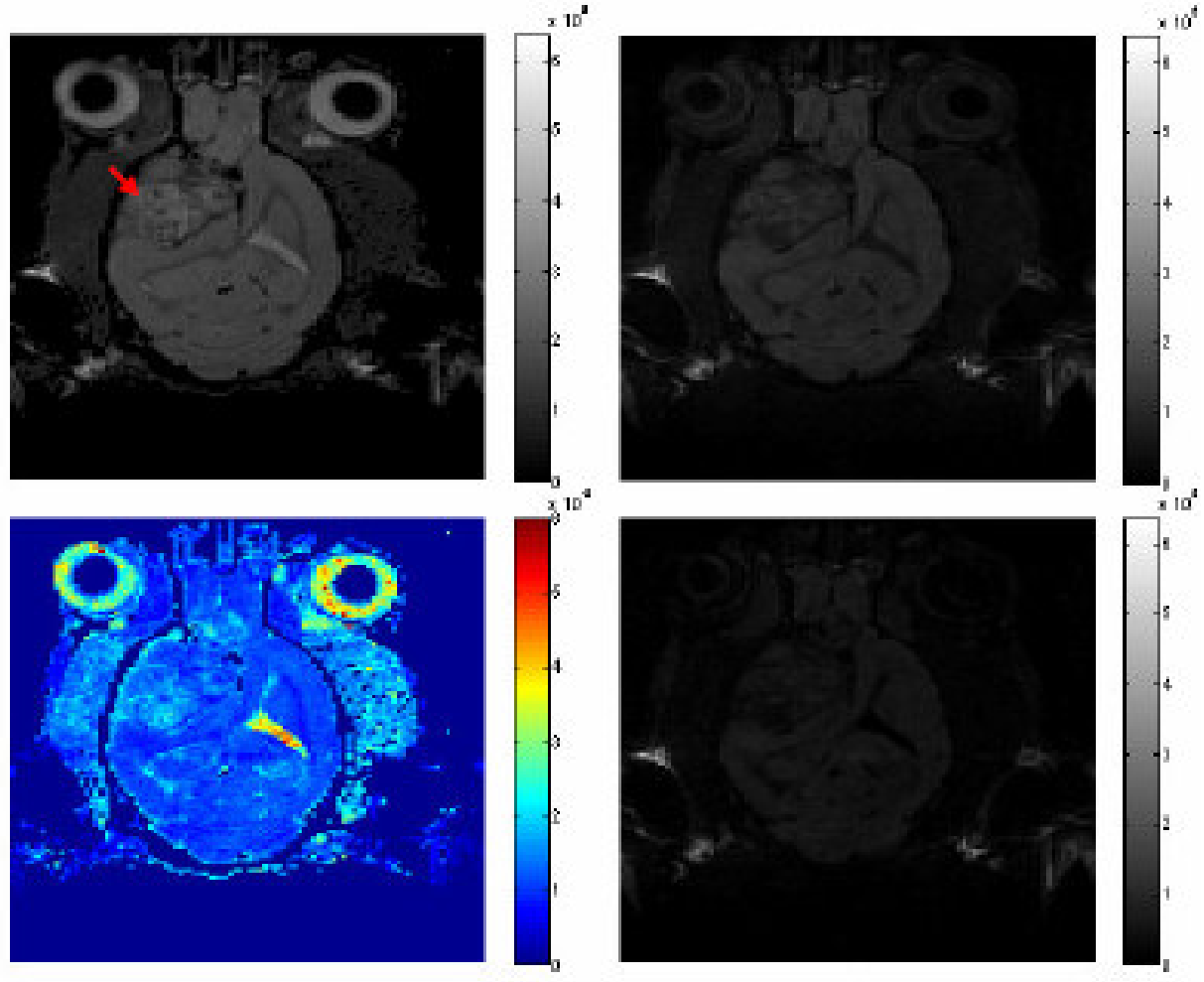

Figure 1: Representative diffusion-weighted MR images and ADC map for a rat with a C6 glioma tumor. Clockwise from top left: MR images acquired with a 9.4 T Varian Inova MR scanner and the corresponding ADC map (units: $mm^2/s$). The location of the glioma is indicated by the red arrow (top left) and is visible in all three MR images as a relatively large, asymmetric region of heterogeneous signal intensity. The glioma is also observable as a region of variable diffusion rates in the ADC map.

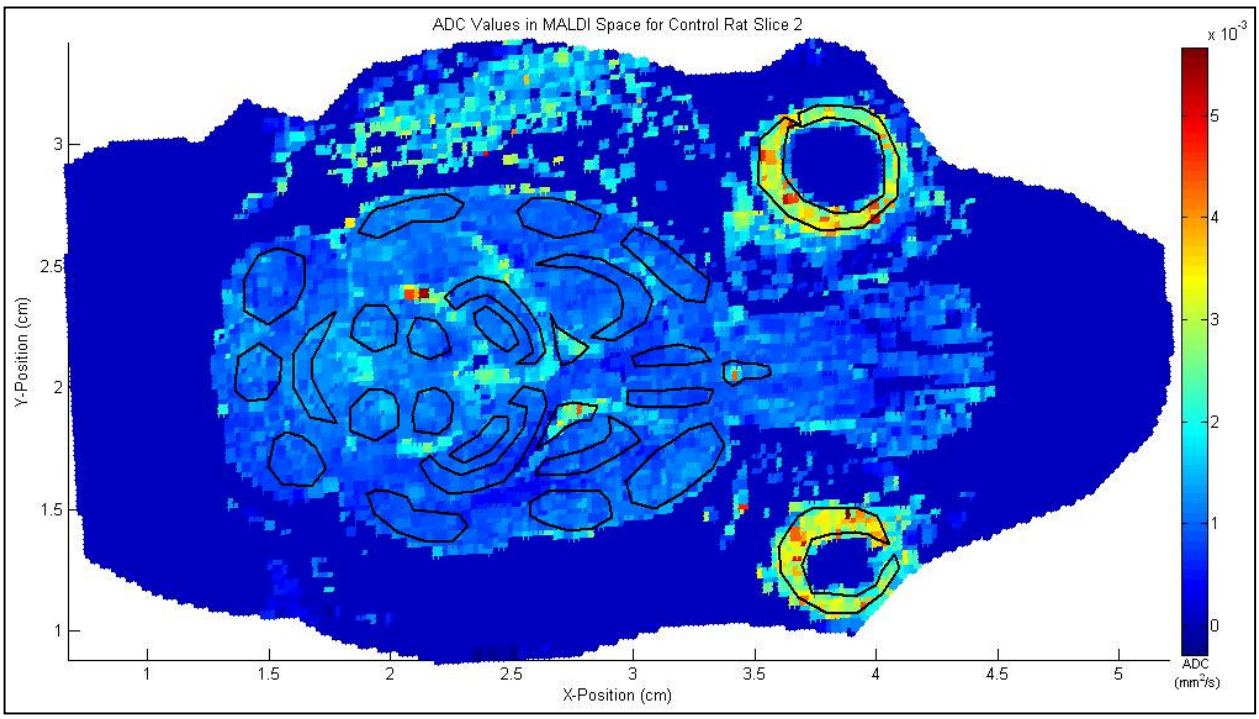


Figure 2: Representative regions of interest in ADC data co-registered to MALDI space. Regions of interest were manually delineated to define selected anatomical structures described in [5].

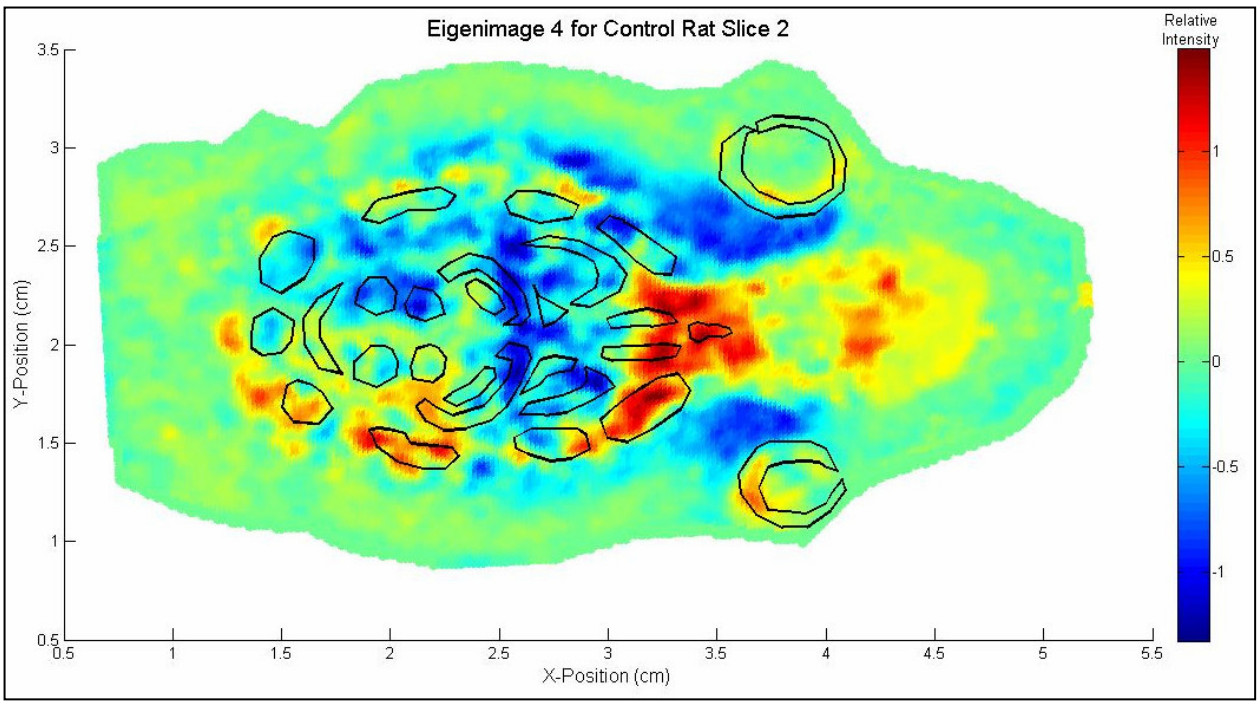

Figure 3: Regions of interest defined in Figure 2 shown within a co-registered eigenimage generated via principal component analysis. The eigenimage represents the relative concentration of MALDI data at each image point.

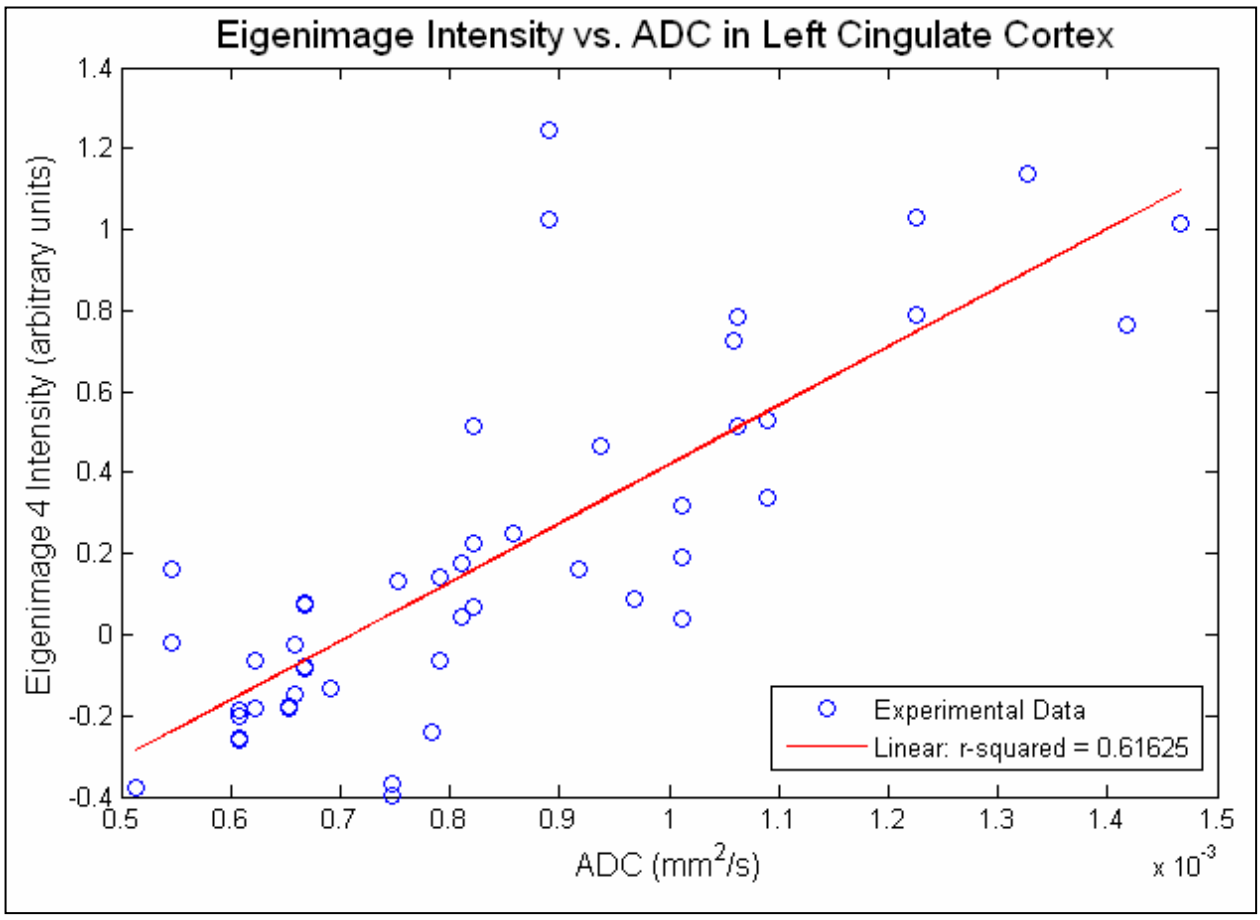


Figure 4: Scatter plot characterizing the relationship between the ADC and eigenimage intensity in a region of interest delineating the left cingulate cortex. The data share an approximately linear relationship with a Pearson correlation coefficient of 0.7850 at a confidence level of 100% (to five significant digits).